United States
Environmental Protection
Agency

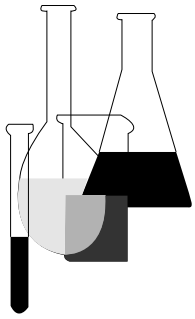
Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–147
April 1996

EPA

Ecological Effects Test Guidelines

OPPTS 850.3020 Honey Bee Acute Contact Toxicity



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 850.3020 Honey bee acute contact toxicity.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C.136, et seq.).

(2) **Background.** The source material used in developing this test guideline is OPP 141–1 Honey Bee Acute Contact LD50 (Pesticide Assessment Guidelines, Subdivision L—Hazard Evaluation; Nontarget Insects) EPA report 540/09-82-019, 1982.

(b) **Purpose.** This guideline is designed to develop data on the acute contact toxicity to honey bees of chemical substances subject to environmental effects test regulations. The Agency will use these and other data to assess acute hazards to bees.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. In addition, the following definitions apply to this guideline:

LD50 is the empirically derived dose of the test substance that is expected to result in mortality of 50 per cent of a population of bees which is treated with a single contact dose under the conditions of the test.

Test substance is the specific form of a chemical or mixture of chemicals that is used to develop the data.

(d) **Test procedures**—(1) **Summary of test.** (i) Test bees may be obtained directly from hives or from frames kept in an incubator. The dosage levels for the definitive test are established, possibly requiring a range-finding test to be conducted first. Test bees are immobilized and randomly assigned to the various dosage levels and controls. Test substance is administered as a single topical dose, either via microapplicator (topical drop) or via whole body exposure to impregnated dust. Bees are closely monitored within the first 4 h after treatment, and then observed for mortality and signs of intoxication at 24 and 48 h. The mortality pattern is examined and subjected to the appropriate statistical analysis to derive the LD50 and confidence limits. The complete mortality pattern, along with signs of intoxication, should be reported.

(ii) A test is unacceptable if more than 20 per cent of the control bees die during the test.

(2) **Range-finding test.** If the approximate toxicity of the test substance is unknown, a range-finding test may be conducted to determine the dosage levels of the test substance to be used in the definitive test. Refer to paragraph (d)(3)(iv) of this guideline for details on dosage levels for definitive tests. If a test substance is expected to be of low toxicity, it may be useful to conduct a limit test at 25 μg per bee first under para-

graph (d)(3)(iv)(B) of this guideline. If mortality occurs at this level, then further range-finding at lower levels will be necessary. The results of the range-finding test may then be used to establish the definitive test dosage levels.

(3) **Definitive test**—(i) **Administration of test substance.** (A) On the day of test initiation, young bees should be collected from the incubator or directly from the hive, immobilized with CO_2 or N_2, and placed in holding cages. To initiate the test, bees in the holding cages are again immobilized, and distributed into groups of at least 25 bees. A single dose will be applied to each bee via microapplicator. Alternatively, test bees may be treated via a dusting apparatus. A minimum of 25 bees is needed for each treatment and control.

(B) A solvent is generally used to administer the test substance. The solvent of choice is acetone, although other volatile organic solvents have been used successfully in cases where acetone was not suitable. Maximum dosage volume should not exceed 5 μL per bee, to allow for adequate volatilization of the solvent.

(C) If a dusting apparatus is used, a nontoxic dust diluent will be required as a carrier.

(ii) **Controls.** (A) Two concurrent controls are required during the test: A negative control and a solvent (or carrier) control. Control bees should be from the same source as the test groups. Control and test bees should be kept under the same environmental conditions. The test procedures should be the same for control and treated bees, except that negative controls receive no treatment and solvent (carrier) controls are treated only with the solvent (carrier). Solvent control bees should receive a volume of solvent equal to the largest volume administered to the test bees. The use of shared controls is acceptable for concurrent tests as long as the same solvent or carrier is used for all the tests.

(B) A test is not acceptable if more than 20 percent of the control bees die during the test period.

(C) A concurrent positive control with a substance of known toxicity is not required. However, a quarterly or semiannual test with a laboratory standard (reference toxicant) is recommended as a means of detecting possible interlaboratory or temporal variation. A laboratory standard is also recommended when there is any significant change in source of bees.

(iii) **Number of animals tested.** (A) In the definitive test, a minimum of 25 bees should be used for each dosage level and for each control. Bees at a treatment level may be divided into replicates if desired.

(iv) **Dosages and dosage-mortality data.** (A) A minimum of five dosage levels of the test substance should be used in the definitive test.

These levels should be spaced geometrically. The recommended spacing is for each dosage level to be at least 60 percent of the next higher level. Ideally, dosage levels should be spaced so that at least three levels result in mortality between 0 and 100 percent.

(B) For test substances expected to have relatively low toxicity, a limit test may be conducted at 25 μg per bee. The LD50 may be reported as greater than 25 μg per bee if 20 bees are dosed at 25 μg per bee, if no mortality occurs, and if test procedures, number of controls, and duration are the same as in a definitive test. Signs of intoxication should be reported. If no mortality occurs, further testing is not required.

(v) **Duration of test.** The definitive test consists of the administration of the test substance followed by an observation period of 48 h.

(vi) **Observations.** (A) Bees should be observed for mortality and toxicological responses at approximately 4, 24, and 48 h after dosing. Dead bees should not be removed from the test chambers until the test is terminated.

(B) All signs of intoxication, other abnormal behavior, and mortality should be recorded throughout the test period and reported by dosage level and by time of occurrence. Signs of intoxication are those behaviors apparently due to the test chemical and may include a wide variety of behaviors, such as ataxia, lethargy, and hypersensitivity. All signs of intoxication and any other abnormal behavior, that may or may not be attributed to the test substance, should be reported.

(4) **Analytical measurements**—(i) **Statistical analysis.** (A) The data should be analyzed by probit analysis, moving average, or binomial probability. The LD50 value, 95 percent confidence limits, and slope of the dose-response curve should be determined for mortality at the end of the test.

(B) All methods used for statistical analysis should be described.

(e) **Test conditions**—(1) **Test species**—(i) **Selection.** (A) Honey bee, *Apis mellifera*, is the test species. Bees may be obtained from on-site colonies or from a commercial apiary. All control and treatment bees used in a test should be from the same source.

(B) Bees used in the test should be in apparent good health. Only bees from disease-free colonies should be used, and they should be kept in conditions conforming to proper cultural practices.

(C) Test should be conducted on worker bees 1– to 7–days–old at test initiation. No acclimation period is necessary. Bees used in the test should be assigned randomly to treatments and controls.

(D) During holding and testing, bees should be shielded from excessive activity or other disturbance. Bees should be handled only as much as is necessary to conform to test procedures.

(ii) **Diet.** A 50 percent sugar/water solution should be provided ad libitum throughout the holding and test periods. Purified or distilled water should be used for the sugar solution.

(2) **Facilities.** (i) Tests should be conducted indoors with bees being maintained in small test chambers. Test chambers may be constructed of metal, plastic, wire mesh, or cardboard, or a combination of these materials. Chambers must be constructed so that a vial containing sugar water may be attached.

(ii) Testing is done indoors to control lighting and other environmental variables. Temperature should be maintained between 25 and 35 °C, with relative humidity between 50 and 80 percent. It is recommended that test bees be maintained in the dark except during dosing and observations.

(f) **Reporting.** The report should include, but not necessarily be limited to, the following information:

(1) Name and address of the facility performing the study and the dates of the study.

(2) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.

(3) Statistical methods employed for analyzing the data.

(4) The test and, if used, control substances identified by name, Chemical Abstracts Service (CAS) number or code number, source, lot or batch number, strength, purity, and composition or other appropriate characteristics.

(5) A description of the methods used, including:

(i) Description of housing conditions, including type, size, and material of pens, and the approximate test room temperature, humidity, and lighting.

(ii) Methods of assigning bees to test chambers.

(iii) Frequency, duration, and methods of observations.

(6) A description of the test system used, including the scientific name of the test species, number used, condition, age at test initiation, and source of test bees.

(7) A description of the dosages, numbers of bees and replicates per dose, and method and time of administration. The reported results should include:

(i) The results of range-finding tests, if conducted.

(ii) For the definitive test, a description of signs of intoxication and other abnormal behavior, including time of onset, duration, severity, and number affected at each dose level and control.

(8) A description of all circumstances that may have affected the quality or integrity of the data.

(9) The name of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(10) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. Results of the analysis of data should include the calculated LD50 value, 95 percent confidence limits, slope of the transformed dose-response line, and the results of a goodness-of-fit test.

(11) The signed and dated reports of each of the individual scientists or other professionals involved in the study, including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(12) The locations where all raw data and the final report are stored.

(13) The statement prepared and signed by the quality assurance unit.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Atkins, E.L., Jr. et al. Equipment and technique used in laboratory evaluation of pesticide dusts in toxicological studies with honey bees. *Journal of Economic Entomology* 47: 965–969 (1954).

(2) Atkins, E.L. et al. Toxicity of pesticides and other agricultural chemicals to honey bees: Laboratory studies. University of California, Division of Agricultural Sciences, Leaflet 2287, 38 pp. (1975).

(3) Finney, D.J. *Probit Analysis*. 3rd ed., Cambridge, London and New York (1971).

(4) Stephan, C.E. Methods for calculating an LC50. Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, American Society for Testing and Materials. Philadelphia, PA. pp. 65–84 (1977).

(5) Stevenson, J.H., Laboratory studies on the acute contact and oral toxicity. *Annals of Applied Biology* 61: 467–472 (1968).

(6) U.S. Environmental Protection Agency, Standard Evaluation Procedure, Honey Bee—Acute Contact LD50 Test. Report No. EPA–540/9–85–002 (1985).